

REMARKS/ARGUMENTS

Claims 1-2 and 4-62 are pending.

Claims 1-2, 29-30, 35, 38-39, 47, 49-50, and 58 have been amended.

Claim 3 has been cancelled.

Claims 10-14 and 17-18 have been withdrawn.

Claim 62 has been added.

Support for the amendments is found in the claims and specification (e.g., the sequences of SEQ ID NO: 1-14), as originally filed. Specifically, Applicants have compared the sequences of SEQ ID NO: 1-14. Based on the comparison, the amino acids in the sequence (I) of claim 1 have been divided in 3 different groups:

- Group 1 : the ones which always represent a polar amino acid selected from the group consisting in Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Lys, Orn, Pro, Ser, Thr and Tyr (these amino acids are J¹, J³, J¹³, J²¹, J²⁷, J³¹, J³³, J³⁴, J³⁶, J⁴⁵, J⁴⁹, J⁵¹, J⁶¹, J⁶³, J⁶⁶, J⁷⁴ and J⁷⁵ in the sequences of SEQ ID NO: 1-14;

- Group 2 : the ones which do not represent such a polar amino acid (these amino acids are J²⁶, J⁶⁴, J⁶⁹ and J⁷¹) in the sequences of SEQ ID NO: 1-14;

- Group 3 : the ones which can represent such a polar amino acid but can also represent another amino acid (these amino acids are J², J⁴, J⁵, J⁶, J⁹, J¹⁰, J²³, J²⁴, J²⁸, J³⁰, J³⁵, J³⁹, J⁴¹, J⁴², J⁴³, J⁴⁶, J⁴⁷, J⁴⁸, J⁵³, J⁵⁴, J⁶⁷, J⁷⁰, and J⁷³) in the sequences of SEQ ID NO: 1-14.

The features of Groups 1 and 2 have been introduced in Claim 1. In addition, as the amino acids J¹⁴, J³⁸, J⁶² and J⁷⁵ always represent an identical amino acid in the sequences of SEQ ID NO: 1-14, this feature is also present in amended Claim 1. The amino acids of Group 3 have been introduced in claim 2.

In new Claim 62, each amino acid J^x is defined by a Markush group corresponding to the different amino acids at position x in the sequences of SEQ ID NO: 1-14.

No new matter is believed to have been added.

Applicants wish to thank the Examiner for indicating the allowable subject matter of claims 4-5, 8-9, and 36-39. Claims 4-5, 8-9, and 36-39 have been objected as being dependent from the rejected claims. However, **claims 4-5 and 8-9 are independent claims.** Claims 36-39 depend from independent claims 4 and 5. **Thus, it is believed that claims 4-5, 8-9, and 36-39 are allowable without additional amendments.**

The present application is a national stage of the PCT/FR03/02025 application, filed June 30, 2003, which claims priority to the applications FR 0208202, filed July 1/2002. A certified copy of FR 0208202 was submitted to the International Bureau in the PCT/FR03/02025 application. A Request for priority under 35 U.S.C. 119 and the International Convention has been submitted with the present application. **Applicants request again granting benefit of the filing date of the priority applications.**

Applicants have filed drawing with the application. However, the drawings have not been accepted or rejected. **Applicants request again that the Examiner indicates acceptance of the drawings.**

Claims 1-3, 6-7, 15-16, 19-35, and 40-61 are rejected under 35 U.S.C. 112, first paragraph, for lack of written description. Applicants respectfully traverse because:

- (a) the amino acids in the peptide sequences (I) and (I') are clearly identified;
- (b) the amino acids are identified at the positions involved in the affinity for phospholipids, toxicity, thermodynamic stability and reversibility of their folding processes;

and

(c) the claimed peptides solve the technical problem of the present invention, i.e., the peptides have improved affinity for phospholipids, toxicity, thermodynamic stability and reversibility of their folding processes.

The purpose of the written description requirement is to ensure that a patent application conveys to a person of skill in the art that the applicants had possession of the claimed invention. *See, e.g., LizardTech, Inc. v. Earth Resource Mapping, Inc.*, 424 F3d 1336, 1345, 76 USPQ2d 1724, 1731 (Fed. Cir. 2005).

The present invention concerns peptides possessing improved affinity for phospholipids, toxicity, thermodynamic stability and reversibility of their folding processes when compared to prior art peptides such as the peptides disclosed in the article of Montaville et al, 2002, JBC, vol. 277, pages 24684-93 (previously submitted). *See* the present specification pages 1-4.

The peptide sequence of claim 1 folds up in space so as to adopt its tertiary conformation, which is the active form of the peptide. Amino acids 12, 15, 16, 17, 19, 20, 22, 50, 55, 57, 58, 59, 60 and 65 are directly or indirectly involved in the binding to lipids, i.e. they are involved either in the three-dimensional structure of the peptide so that it adopts its active conformation allowing recognition of a negatively charged lipid, or in the peptide recognition site. *See* the present specification pages 3-8.

The amino acids J are the surface amino acids of this peptide when it is in its folded and active conformation. These residues are arranged spatially such that they are partially or completely exposed to the solvent. The amino acids J are selected from all the natural amino acids and at least 50% of them are polar residues selected from Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Lys, Orn, Pro, Ser and Thr. *See* the present specification page 6.

Further, the Examiner has pointed out in the Official Action on page 5 that the disclosed peptides share homology amongst one another. Applicants have compare the

sequences of SEQ ID NO: 1-14 and divided the amino acids of the sequences in 3 different groups:

Group 1 : the amino acids that always represent a polar amino acid selected from the group consisting in Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Lys, Orn, Pro, Ser, Thr and Tyr (these amino acids are J¹, J³, J¹³, J²¹, J²⁷, J³¹, J³³, J³⁴, J³⁶, J⁴⁵, J⁴⁹, J⁵¹, J⁶¹, J⁶³, J⁶⁶, J⁷⁴ and J⁷⁵);

Group 2 : the amino acids that do not represent such a polar amino acid (these amino acids are J²⁶, J⁶⁴, J⁶⁹ and J⁷¹); and

Group 3 : the amino acids that can represent such a polar amino acid but can also represent another amino acid (these amino acids are J², J⁴, J⁵, J⁶, J⁹, J¹⁰, J²³, J²⁴, J²⁸, J³⁰, J³⁵, J³⁹, J⁴¹, J⁴², J⁴³, J⁴⁶, J⁴⁷, J⁴⁸, J⁵³, J⁵⁴, J⁶⁷, J⁷⁰, and J⁷³).

The features concerning group 1 and group 2 have been introduced in amended Claim 1. In addition, as the amino acids J¹⁴, J³⁸, J⁶² and J⁷⁵ always represent an identical amino acid in sequences SEQ ID NO: 1-14, this feature is also present in amended Claim 1.

As the sequence (I) contains 47 amino acids J, the requirement that at least 50% of amino acids J are polar residues selected from the group consisting of Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Lys, Orn, Pro, Ser, Thr and Tyr means that at least 24 amino acids J must be selected amongst these polar groups. One skilled in the art knows from Group 1 the precise localization of 19 of these polar amino acids J and also knows that the last 5 polar amino acids cannot be selected in Group 2 but must be selected among the other amino acids which constitute Group 3.

The amino acids U are the core residues of the claimed peptide. In the folded and active conformation of the peptide, they are spatially arranged close to one another and are not exposed to the solvent. They constitute the hydrophobic core of the protein. The compact assembly of the atoms of these residues plays a predominant role in the stability of the peptide in its active conformation. The amino acids U at each identified position are selected

from a limited number of residues (at most 5). *See* the present specification pages 6-7.

Further, in Claim 1 the peptides comprise the sequence (I) with amino acids U as defined in Table 1 and the specific amino acids at positions 7, 12, 14, 16, 17, 19, 20, 22, 32, 37, 38, 50, 55, 57, 58, 60, 62 and 75 as defined in the sequences of SEQ ID No: 1-14 of the sequence listing.

The function of the residue X¹⁸ is to maintain the structure of the Gly-X-Gly loop in the active form of the peptide, in particular where the residues Z⁵⁹ and Z⁶⁵ are Glu, to modulate the hydrophobic and lipophilic nature of this loop, and to provide new specific interactions with phospholipids. This is the case, for example, of the residues Asn, Cys, Ser, Thr, Trp and Tyr. *See* the present specification page 7.

The residues Z⁵⁹ and Z⁶⁵ are advantageously lysine residues, the effect of which is to replace the calcium ion with the positively charged -NH₃⁺ group of the lysine and to improve the affinity of the peptide for a negatively charged membrane. *See* the present specification pages 7-8.

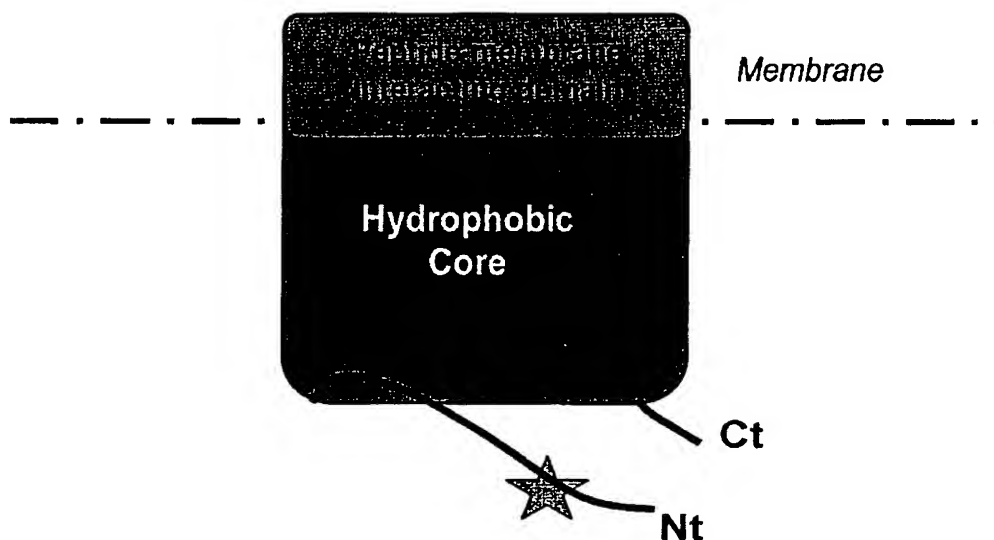
The peptide of the sequence (I), in its active form, comprises three sites for binding to a calcium ion where the calcium ion complexed with this site constitutes one of the ligands of a negatively charged phospholipid. The first of these sites, called principle site, involves residues 15, 18, 19 and 59 as calcium ligands. The second of these sites, called secondary site, involves residues 20 and 22 as calcium ligands. The third of these sites, which is a low-affinity secondary site, involves residues 57, 60 and 65 as calcium ligands. Thus, the residues involved overall in the binding to phospholipids are residues 12, 15, 16, 19, 20, 22, 50, 55, 57, 58, 69, 60 and 65. This list includes residues involved in calcium binding, the phospholipids being calcium ligands. *See* the present specification pages 8-11.

Because of the improved properties and the labeling, the claimed peptides are useful for detecting not only *in vitro* but also *in vivo*, e.g., apoptotic cells or foci and negatively

charged lipids at the surface of the cells. *See* the present specification, pages 1-3, the Examples.

Thus, the scope of the peptide sequence of Claim 1 has been restricted so that the amino acids are clearly identified.

The identified positions are fundamental in order to solve the technical problem of the present invention. The claimed peptide can be presents by the following diagrammatic structure:



As explained at pages 6 and 8 of the present specification, the domain directly or indirectly interacting with the membrane lipids comprises the residues 12, 15, 16, 17, 19, 20, 22, 50, 55, 57, 58, 59, 60 and 65. These residues affect the peptide affinity for lipids and are clearly identified in the peptide sequence of the Claim 1.

The stability and more generally the thermodynamic properties of the claimed peptide depend on the “hydrophobic core” domain the residues of which are the residues U and B listed in Table 1. To improve the properties in comparison with annexin, it is necessary to

select a suitable combination of hydrophobic residues, but the solution is not, however, unique and Table 1 includes combinations deemed best.

The lower part of the claimed peptide (see the drawing above) comprises, in particular, N-terminal and C-terminal segments to be used for various labels (for example, positioned at the star of the diagram) and/or grafting on various supports.

For surface residues of the claimed peptide according to the invention, other than those mentioned above, there is a certain freedom of choice. It should be noted however that some of these amino acids were set in the peptide sequence of the amended Claim 1 and on the basis of amino acids routinely found in the same position in SEQ ID NO. 1 to 14 of the appended sequence listing.

Applicants respectfully point out to the Examiner that the term “derivatives” for the amino acids permitted for variable J has been already deleted from Claim 1 and the variable B³⁷ is limited to Arg.

In new Claim 62, each amino acid J^x is defined by a Markush list corresponding to the different amino acids at position x in the sequences SEQ ID NO: 1-14.

Thus, it is possible to define more precisely the other residues and mainly surface residues. In particular, among these residues, the localization of the polar amino acids that are selected from the group consisting of Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Lys, Orn, Pro, Ser, Thr and Tyr has been defined more precisely.

The peptide sequence (I) of Claim 1 has clearly identified amino acids. Also, the amino acids are identified at the positions involved in the affinity for phospholipids, toxicity, thermodynamic stability and reversibility of their folding processes.

It is therefore clear that the peptides comprising the peptide sequence as defined in Claims 1 and 62 can solve the technical problem of the present invention.

Thus, it is believed that the specification provides an adequate description for the genus of the claimed peptide.

Applicants request that the rejection be withdrawn.

Claims 1-2, 6-7, 15-16, 19-35, and 40-61 are rejected under 35 U.S.C. 112, second paragraph. The claims have been amended to clarify variables within the sequence. It is believed that the claims are clear. Applicants request that the rejection be withdrawn.

A Notice of Allowance for all pending claims is requested.

Respectfully submitted,

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